Fourier transform infrared spectroscopy enables rapid differentiation of fresh and frozen/thawed chicken

Grunert, Tom ; Stephan, Roger ; Ehling-Schulz, Monika ; Johler, Sophia

Abstract: Freezing and thawing affect the sensory profile and the quality of chicken meat, resulting in lower marketability. Retailers are faced with the risk of mislabeling, as fresh and frozen/thawed chicken meat are visually indistinguishable and as there is currently no fast, reproducible, and inexpensive technique for the differentiation of fresh and frozen/thawed chicken implemented in practice. Fourier Transform Infrared (FTIR) spectroscopy represents a new promising technique that determines the overall chemical composition of a sample, thus creating a metabolic spectral fingerprint that can be analyzed by various pattern recognition algorithms. In this study, we aimed to assess the performance of FTIR spectroscopy when applied to the differentiation of fresh and frozen/thawed chicken meat. To this end, we compared the FTIR spectra of chicken stored at 4 °C to those of chicken that was frozen and stored at −20 °C for 2, 5, 15, 30, 60, 70, and 85 days. Hierarchical cluster analysis of FTIR spectra allowed to distinguish fresh samples from samples that have been frozen for longer periods. Samples of frozen storage of 15, 30, 75 and 85 days could be clearly identified as such. Further, the potential of combining FTIR spectroscopy with artificial neuronal network (ANN) analysis to enable identification of even shortly frozen products was determined. Twenty out of 21 samples were correctly classified in either fresh or frozen/thawed chicken meat based on the internal validation including frozen/thawed chicken meat samples derived from day 2 and 5. In conclusion, we provide proof of principle that FTIR spectroscopy enables rapid and reliable discrimination of fresh from frozen/thawed chicken meat. Due to its high–throughput capacity, it could represent a promising tool in routine inspections differentiating fresh from previously frozen meat products such as beef, pork, lamb and turkey.

DOI: https://doi.org/10.1016/j.foodcont.2015.08.016

Short communication

Identification of frozen/thawed chicken by FTIR spectroscopy

Fourier Transform Infrared Spectroscopy Enables Rapid Differentiation of Fresh and Frozen/Thawed Chicken

Tom Grunert[a,*], Roger Stephan[b], Monika Ehling-Schulz[a], Sophia Johler[b]

[a]Functional Microbiology, Institute of Microbiology, University of Veterinary Medicine Vienna, Veterinaerplatz 1, 1210 Vienna, Austria

[b]Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, Winterthurerstrasse 272, 8057 Zurich, Switzerland

Key words: FTIR, chicken meat, freezing, refrigeration, mislabeling

*Author for correspondence. Tel: +43-1-250772469; Fax: +43-1-250772479 E-mail: tom.grunert@vetmeduni.ac.at

ABSTRACT

Freezing and thawing affect the sensory profile and the quality of chicken meat, resulting in lower marketability. Retailers are faced with the risk of mislabeling, as fresh and frozen/thawed chicken meat are visually indistinguishable and as there is currently no fast, reproducible, and inexpensive technique for the differentiation of fresh and frozen/thawed chicken implemented in practice. Fourier Transform Infrared (FTIR) spectroscopy represents a new promising technique that determines the overall chemical composition of a sample, thus creating a metabolic spectral fingerprint that can be analyzed by various pattern recognition algorithms. In this study, we aimed to assess the performance of FTIR spectroscopy when applied to the differentiation of fresh and frozen/thawed chicken meat. To this end, we compared the FTIR spectra of chicken stored at 4°C to those of chicken that was frozen and stored at -20°C for 2, 5, 15, 30, 60, 70, and 85 days. Hierarchical cluster analysis of FTIR spectra allowed to distinguish fresh samples from samples that have been frozen for longer periods. Samples of frozen storage of 15, 30, 75 and 85 days could be clearly identified as such. Further, the potential of combining FTIR spectroscopy with artificial neuronal network (ANN) analysis to enable identification of even shortly frozen products was shown. Twenty out of 21 samples, were correctly classified in either fresh or frozen/thawed chicken meat based on the internal validation including frozen/thawed chicken meat samples derived from day 2 and 5. Since, we provide new data to underscore the potential of applying FTIR spectroscopy to rapidly and reliably distinguish fresh and frozen/thawed chicken meat, it could be applied by both retailers and governmental control agencies to ascertain correct labeling of chicken meat. In the future, a larger set of meat samples derived from several different meat producers should be used to train a robust pattern recognition algorithm, such as the presented ANN, for the FTIR spectroscopic identification of previously frozen products, including beef, pork, lamb and turkey.

1. Introduction

Chicken is a highly perishable meat product. Freezing and frozen storage of chicken and other meat products is a common practice used in the striving global meat export industry, which is currently worth more than 13 billion USD (Leygonie et al., 2012). According to the Food and Agriculture Organization of the United Nations, freezing of poultry can extend the practical storage life to up to two years (Cano-Muñoz, 1991). However, freezing and thawing negatively affects the sensory profile and therefore the quality of meat through formation of ice crystals, oxidation of lipids and degradation of proteins, reduced tenderness, and reduced water holding capacity (Ali et al., 2015; Leygonie et al., 2012). The impaired quality of frozen/thawed chicken and the resulting impact on marketability is also reflected in lower pricing. In December 2014, the EU implemented a new regulation specifying that frozen/thawed products have to be labeled "defrosted", as safety, taste and the physical quality of food items – in particular meat and fish – could be affected (EU Regulation 1169/2011). However, fresh and frozen/thawed chicken meat are virtually indistinguishable. In addition, most of the the currently available testing methods for differentiation of fresh and frozen/thawed chicken are laborious, time consuming, and cost intensive (Bae, 2014). These include enzymatic methods, DNA based techniques, spectroscopic methods using light in the ultraviolet, visible (UV-VIS) and near-infrared (NIR) electromagnetic spectrum, bio imaging and sensory methods (as reviewed in Ballin et al., 2008; Ballin et al. 2010; Jung et al., 2011). Consequently, retailers often rely on information provided by sub-contractors, which bears the risk of mislabeling and its negative impact on the consumers' repurchase behavior. Therefore, a novel high-throughput tool suited for reliable differentiation of fresh and frozen/thawed chicken is urgently needed.

Fourier Transform Infrared Spectroscopy (FTIR) is a new promising tool that measures the overall chemical composition of a sample, thus creating a metabolic spectral fingerprint that

can be analyzed by various pattern recognition algorithms (Wenning & Scherer, 2013). It is successfully employed as an analytical tool in a wide range of fields and industries including microbiological and medical diagnostics, as well as food science and technology (Naumann, 2008; Rodriguez-Saona & Allendorf, 2011). In the late nineties, FTIR spectroscopy was used to study meat from pork, chicken, and turkey after frozen storage at day 15 (Al Jowder et al., 1997). However, this study used minced meat, which is sold for a lower price than intact muscle meat. It shows different freezing properties and is increasingly more susceptible to food spoilage as well as degradation processes compared to muscle meat. Therefore, in this study we aimed to compare FTIR spectra of fresh and frozen/thawed intact muscle chicken meat in order to evaluate the potential of the application of FTIR spectroscopy as a tool for identification of frozen storage of chicken meat.

2. Materials and methods

2. 1. Chicken meat samples

An overview of all chicken meat samples used is provided in Table 1. A total of 16 samples of 50 g of chicken breast muscle were purchased from a local poultry meat producer (producer A). The fresh meat was immediately packed in plastic bags in line with the industry standard and vacuum-sealed. The cooled samples (4°C) were transported to the laboratory. The samples were randomly assigned to two groups: fresh samples that were only refrigerated (R; n = 6), and frozen/thawed samples (FT; n = 10). Refrigerated samples were kept at 4°C and were prepared for FTIR spectroscopy immediately (T_0) and after 2 and 5 days. The frozen/thawed samples were stored at -20°C for 2, 5, 15, 30, 60, and 70 days and were gently thawed at 4°C for 6-7 h prior to sample preparation for FTIR spectroscopy. Two samples

from a different poultry meat producer (producer B) were used for validation and were frozen for 85 days prior to sample preparation for FTIR spectroscopy.

2.2. Sample preparation and FTIR spectroscopy.

We transferred 46.9 ± 1.09 (mean ± SD) grams of the samples to 50 mL polypropylene centrifuge tubes. Upon centrifugation at 15,000 *x g* for 15min at 4°C, the aqueous phase ("press-juice") was removed and triplicates of 1:10 and 1:15 dilutions in 0.9% NaCl were prepared for two samples of each group and time point. A volume of 30 µl of the press-juice dilutions was spotted on a zinc selenite (ZnSe) optical plate and dried at 40°C for 30 min. Infrared spectroscopy absorption spectra were recorded using a HTS-XT microplate adapter coupled to a Tensor 27 FTIR spectrometer (Bruker Optics GmbH, Ettlingen, Germany). Spectral acquisition was performed in transmission mode in the spectral range of 4,000 to 500 cm^{-1} using the following parameters: 6 cm^{-1} spectral resolution, zero-filling factor 4, Blackmann-Harris 3-term apodization and 32 interferogramms were averaged with background subtraction for each spectrum (Grunert et al., 2014).

2.3. Spectral processing and chemometrics.

Spectral evaluation and processing were performed using the software OPUS 7.2 (Bruker Optics GmbH). Second derivatives of the original spectra with a 9-point Savitzky-Golay filter were calculated to increase spectral resolution and to minimize problems with baseline shifts. Subsequent vector normalization was performed for the whole spectral range to adjust biomass variations among different sample preparations (Grunert et al., 2014). Subtractive spectral analysis was used to define spectral regions of critical relevance to the discrimination of the different experimental groups. An average spectrum was calculated from the recorded, second derivative and vector normalized IR spectra of the R and FT group separately and

differential spectra analysis was performed. The average spectrum of R samples was subsequently subtracted from the average spectrum of FT samples. Chemometric analysis was performed on preprocessed data employing unsupervised hierarchical cluster analysis (HCA). Unsupervised methods are not based on prior knowledge and allow reduction of data complexity, while maintaining most of the original variance (Wenning & Scherer, 2013). The spectral window 1660-1628 cm^{-1}, was selected for HCA, offering the maximum discriminatory power.

To develop and to validate the potential of an artificial neuronal network (ANN) for the discrimination between fresh and frozen/thawed chicken meat, the software NeuroDeveloper (version 2.5b; SynthonGmbH, Heidelberg, Germany) was used (Udelhoven et al., 2003). Preprocessed spectra used for HCA (n=108) were subdivided into two groups: (1) 86 spectra served as a reference data set (n = eight to ten spectra/ group) and (2) 21 spectra (n = two to three spectra/ group) were used for internal validation. One spectrum was excluded based on an outlier analysis using the software Unscrampler X (CAMO Software, Oslo, Norway). The reference data set used to calibrate the model was randomly divided into a training set (n = six to eight spectra/ group) and a prevalidation set (n = two spectra/ group). To reduce the dimensionality in the spectral data set, the most discriminative wavenumbers were identified prior to the training process using the multivariate COVAR algorithm of the NeuroDeveloper software (based on a covariance analysis combined with the sequential forward selection search strategy).

3. Results and discussion

We evaluated the performance of FTIR spectroscopy as a tool for the differentiation of fresh (refrigerated only) and frozen/thawed chicken meat. Differential spectral analysis revealed significant differences between R and FT samples within the protein region (1800 - 1500cm^{-1}) at 1,660 - 1,628 cm^{-1} (Fig. 1). Spectral alterations in this frequency area are primarily determined by the confirmation sensitive amide I band (1670 - 1625 cm^{-1}) principally based on symmetric stretching vibrations of the carbonyl (C=O) functional group. The amide I band is indicative for changes in the protein secondary structure including alterations in the backbone conformation and the hydrogen bonding pattern. Subtractive FTIR spectral analysis revealed changes between R and FT samples associated to α-helical (1651 cm^{-1}) and β-plated sheet (1639 cm^{-1}, 1633 cm^{-1}) protein secondary structures.

This highly discriminatory spectral range (1,660 - 1,628 cm^{-1}) was further applied for chemometric analysis by unsupervised HCA. A dendrogram depicting the clustering of the FTIR spectra is provided as Fig. 2.. While FTIR spectra of samples that had only been frozen for up to five days could not be distinguished by HCA from those of fresh chicken samples, longer periods of frozen storage (15, 30, 70, 85 days) could be clearly identified. Thus, these results demonstrate that using HCA alone is insufficient to correctly classify samples of shorter periods of frozen storage.

Therefore, the next steps of the assessment of the use of FTIR spectroscopy for the routine differentiation between fresh and frozen/thawed meat samples should be based on more powerful, supervised learning methods, such as ANNs. Indeed, compared to unsupervised learning methods (e.g. HCA, Principal component analysis-PCA) ANNs have been shown to significantly improve the discriminatory power and are particularly suited for routine analytical purposes, because they enable analysis of “unknown samples” in a fairly straightforward ‘yes’ or ‘no’ manner (see Rebuffo et al., 2006; Lasch et al., 2007; Grunert et

al., 2013). The establishment of the ANN was based on the same second derivative, vectornormalized spectra used for HCA. The ANN was trained with selected spectral signatures defined by the COVAR algorithm as input data (input neurons) paired with the predefined output classes fresh (refrigerated only) and frozen/thawed chicken meat (output neurons) (for details, see Materials and Methods). To achieve an optimal network performance the input and hidden neurons were automatically adjusted during the iterative training process until the global error was at its minimum (Naumann, 2000). The ANN training resulted in a single-level ANN using the 22 most discriminative wavenumbers (input neurons), two hidden neurons, and two output neurons. Two to three randomly selected spectra of each of the nine sample groups ($R_{0,2,5}$ and $FT_{2,5,15,30,75,85}$) were used for internal validation. A correct classification was achieved for 20 out of 21 samples. One sample (FT_{85}) yielded an ambiguous result. FTIR spectroscopy-based differentiation of fresh and frozen/thawed meat requires only 1 hour analysis time in total (including sample preparation and FTIR spectroscopy measurement) and less than 5min 'hands-on time' for preparation per sample. In addition, only a minimum of 5µL "press-juice" per measurement is required. Operating expenses for running the FTIR spectroscopy measurement are low because no further reagents are required and sample holder (ZnSe plates) are reusable.

Various techniques for the differentiation of fresh and frozen/thawed meat and fish products have been described. However, to date, no rapid, reliable, and inexpensive technique for the identification of frozen/thawed chicken has been successfully implemented. Differentiation of fresh and frozen/thawed products is of high interest to retailers not only in the case of poultry, but also for all other kinds of meat (Benjakul & Bauer, 2000). In this study, we provide new data to underscore the potential of applying FTIR spectroscopy to distinguish fresh and frozen/thawed chicken meat. However, ANN-assisted FTIR spectroscopy requires a large spectral data base. Thus, further studies must be performed to collect samples from different

meat species and producers to develop an automated and robust biochemical-based differentiation algorithm based on spectral features which might enable the identification of various previously frozen meat products.

4. Conclusion

This study provides proof of principle that FTIR spectroscopy and subsequent HCA and/ or ANN is suitable to distinguish between refrigerated chicken and chicken that had been subjected to frozen storage. It may represent a promising tool to be used by both retailers and governmental control agencies to ascertain correct labeling of chicken meat. In the future, the use of improved pattern recognition algorithms trained with a larger data set could further improve the performance of FTIR spectroscopic identification of frozen/thawed products, including beef, pork, lamb, and turkey.

207 **REFERENCES**

208 Ali, S., Zhang, W., Rajput, N., Khan, M. A., Li, C., & Zhou, G. (2015). Effect of multiple
209 freeze–thaw cycles on the quality of chicken breast meat. *Food Chemistry*, *173*, 808–
210 814.

211 Al Jowder, O., Kemsley EK, Wilson RH. (1997). Mid-infrared spectroscopy and authenticity
212 problems in selected meats: a feasibility study. *Food Chem.* 59, 195-201.

213 Al-Jowder O., Defernez M., Kemsley EK., Wilson RH. (1999). Mid-infrared spectroscopy
214 and chemometrics for the authentication of meat products. *J Agric Food Chem,*
215 47(8):3210-8.

216 Bae 2012… Lieber Herr Stephan, ich kann diese Referenz leider nicht finden und bitte Sie
217 diese, falls bekannt, zu ergänzen. Habe andernfalls an Sophia schon ein Mail geschickt.
218 Danke.

219 Ballin NZ., Lametsch R. (2008). Analytical methods for authentication of fresh vs. thawed
220 meat - A review. *Meat Sci.* 80(2):151-8.

221 Ballin NZ. (2010). Authentication of meat and meat products. *Meat Sci.,*86(3):577-87.

222 Benjakul, S., & Bauer, F. (2000). Physicochemical and enzymatic changes of cod muscle
223 proteins subjected to different freeze-thaw cycles. *Journal of the Science of Food and*
224 *Agriculture*, *80*, 1143–1150.

225 Cano-Muñoz, G. (1991). Manual on meat cold store operation and management. In *FAO*
226 *animal production and health paper, no.92*, 121.

227 Grunert T, Wenning M, Barbagelata MS, Fricker M, Sordelli DO, Buzzola F, Ehling-Schulz
228 M. (2013). Rapid and reliable identification of *Staphylococcus aureus* capsular serotypes
229 by means of artificial neural network-assisted Fourier transform infrared spectroscopy. *J*
230 *Clin Microbiol* 51: 2261–2266.

231 Grunert, T., Monahan, A., Lassnig, C., Vogl, C., Müller, M., & Ehling-Schulz, M. (2014).
232 Deciphering host genotype-specific impacts on the metabolic fingerprint of *Listeria*
233 *monocytogenes* by FTIR spectroscopy. *PLoS ONE*, *9*, e115959.
234 Jung, S., Lee, J. C., Jung, Y., Kim, M. K., Son, H., & Jo, C. (2011). Instrumental methods for
235 differentiation of frozen-thawed from fresh broiler breast fillets. *Korean J. Food Sci. An.*,
236 *31*, 27–31.
237 Lasch P, Beekes M, Schmitt J, Naumann D. Detection of preclinical scrapie from serum by
238 infrared spectroscopy and chemometrics. (2007). *Anal Bioanal Chem.* 387(5):1791-800.
239 Leygonie, C., Britz, T. J., & Hoffman, L. C. (2012). Impact of freezing and thawing on the
240 quality of meat: Review. *Meat Science*, *91*, 93–98.
241 Naumann D. 2000. Infrared spectroscopy in microbiology, p 102–131. In Meyers RA (ed),
242 Encyclopedia of analytical chemistry. John Wiley & Sons Ltd, Chichester, United
243 Kingdom.
244 Naumann, D. (2008). Vibrational spectroscopy in microbiology and medical diagnostics. In P.
245 Lasch & J. Kneipp (Eds.), *Biomedical Vibrational Spectroscopy* (pp. 1–8).
246 Rebuffo CA, Schmitt J, Wenning M, von Stetten F, Scherer S. (2006). Reliable and rapid
247 identification of *Listeria monocytogenes* and *Listeria* species by artificial neural
248 network-based Fourier transform infrared spectroscopy. *Appl. Environ. Microbiol.*
249 72:994 –1000.
250 Rodriguez-Saona, L. E., & Allendorf, M. E. (2011). Use of FTIR for rapid authentication and
251 detection of adulteration of food. *Annual Review of Food Science and Technology*, *2*,
252 467–483.
253 Udelhoven, T., Novozhilov, M., & Schmitt, J. (2003). The NeuroDeveloper: a tool for
254 modular neural classification of spectroscopic data. *Chemometrics and Intelligent*
255 *Laboratory Systems*, *66*, 219–226.

256 Wenning, M., & Scherer, S. (2013). Identification of microorganisms by FTIR spectroscopy:
257 perspectives and limitations of the method. *Applied Microbiology and Biotechnology*,
258 *97*, 7111–7120.
259

FIGURE LEGENDS

Figure 1: Subtractive FTIR spectral analysis. Second derivate, vector-normalized FTIR average spectra were generated from refrigerated and frozen/thawed chicken samples. Spectra from frozen/thawed chicken samples were subtracted from refrigerated chicken samples. Most profound differences were observed in the highlighted spectral range of 1,660 - 1,628 cm^{-1}, which can be assigned to amide I (1,670 - 1,625 cm^{-1}).

Figure 2: Dendrogram based on 108 FTIR spectra of refrigerated (R) and frozen/thawed (FT) chicken analyzed by HCA. While clusters 3 - 5 comprise FT samples only, clusters 1 and 2 are mixed clusters of spectra of both R and FT samples. Cluster 1 comprises predominantly spectra of R samples (R_0, R_2, R_5; n = 31), but also spectra of FT samples (FT_2, FT_5; n = 4). Cluster 2 comprises predominantly spectra of FT samples (FT_2, FT_5; n = 20), but also spectra of R samples (R_5; n = 5).

TABLES

Table 1: Random assignment of chicken meat samples into two groups: fresh samples that were refrigerated only (R, n = 6); and frozen/thawed samples (FT, n = 12). Two samples per group and time point were analyzed in triplicates of 1:10 and 1:15 dilutions to generate a total of 108 FTIR spectra.

Group	Sample ID	Storage duration	Storage conditions	Number of samples	Sample source
R	R_0	0 days	4°C	2	Producer A
	R_2	2 days	4°C	2	Producer A
	R_5	5 days	4°C	2	Producer A
FT	FT_2	2 days	-20°C	2	Producer A
	FT_5	5 days	-20°C	2	Producer A
	FT_{15d}	15 days	-20°C	2	Producer A
	FT_{30d}	30 days	-20°C	2	Producer A
	FT_{70d}	70 days	-20°C	2	Producer A
	FT_{85d}	85 days	-20°C	2	Producer B